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Placental Blood as a Source of Hematopoietic Stem Cells for Transplantation into Unrelated Recipients

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ABSTRACT

Background Transplantation of bone marrow from unrelated donors is limited by a lack of HLA-matched donors and the risk of graft-versus-host disease (GVHD). Placental blood from sibling donors can reconstitute hematopoiesis. We report preliminary results of transplantation using partially HLA-mismatched placental blood from unrelated donors.

Methods Twenty-five consecutive patients, primarily children, with a variety of malignant and nonmalignant conditions received placental blood from unrelated donors and were evaluated for hematologic and immunologic reconstitution and GVHD. HLA matching was performed before transplantation by serologic typing for class I HLA antigens and low-resolution molecular typing for class II HLA alleles. In donor-recipient pairs who differed by no more than one HLA antigen or allele, high-resolution class II HLA typing was done retrospectively. For donor-recipient pairs who were mismatched for two HLA antigens or alleles, high-resolution typing was used prospectively to select the best match for HLA-DRB1.

Results Twenty-four of the 25 donor-recipient pairs were discordant for one to three HLA antigens. In 23 of the 25 transplant recipients, the infused hematopoietic stem cells engrafted. Acute grade III GVHD occurred in 2 of the 21 patients who could be evaluated, and 2 patients had chronic GVHD. In vitro proliferative responses of T cells and B cells to plant mitogens were detected 60 days after transplantation. With a median follow-up of 12¹/₂ months and a minimal follow-up of 100 days, the overall

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100-day survival rate among these patients was 64 percent, and the overall event-free survival was 48 percent.

Conclusions HLA-mismatched placental blood from unrelated donors is an alternative source of stem cells for hematopoietic reconstitution in children.

Allogeneic bone marrow transplantation can cure some hematologic cancers, bone marrow failure syndromes, immunodeficiency disorders, and inborn errors of metabolism,^{1,2,3,4,5} but its success depends on the prompt identification of a suitable donor and the avoidance of severe graft-versus-host disease (GVHD).^{6,7,8,9,10} Transplantation of hematopoietic stem cells from placental or cord blood can overcome these problems.

Over the past seven years, placental blood from a sibling has been used as a source of hematopoietic stem cells in more than 100 allogeneic transplantations.^{11,12,13,14} In 44 placental-blood transplantations involving sibling donors that were reported to the International Cord Blood Transplant Registry, there was successful hematopoietic reconstitution and a lower incidence of GVHD than expected with bone marrow grafts.^{11,12} Delays in myeloid engraftment were noted, but the probability of event-free survival was 72 percent after a median follow-up of 1.6 years.

In 1992 the Placental Blood Program was established at the New York Blood Center to explore the feasibility of using banked placental blood from unrelated donors for the transplantation of hematopoietic stem cells.¹⁵ In this report we describe the preliminary results of 25 consecutive transplantations of placental blood from unrelated donors that were performed at a single center with units obtained through the Placental Blood Program. Nearly all of the patients in this study were children (age range, 0.8 to 23.5 years).

Methods

Eligibility and Study Objectives

This phase 1 study of the transplantation of placental blood for the treatment of malignant and nonmalignant conditions was approved by the institutional review board of Duke University Medical Center. Patients were eligible for enrollment if there was neither an HLA-identical related donor nor a related donor with two HLA mismatches available and if an HLA-matched, unrelated bone marrow donor could not be identified within six months. Informed consent and a sample of autologous backup bone marrow were also required.

Donor Selection

Beginning in September 1993, formal searches were conducted by the Placental Blood Program on behalf of 210 patients referred to the Duke University Medical Center. Initial matching criteria for an HLA-matched unit required the placental blood to share at least five HLA antigens with the potential recipient. These antigens were identified by serologic typing (HLA class I) and low-resolution DNA typing (HLA class II). Such matched units were found for 92 of the 210 patients (44 percent). Of these 92 units, 6

matched all six of the recipients' class I and class II major histocompatibility complex (MHC) antigens. Donor units that matched with four of the prospective recipients' six class I and II HLA antigens were sought for 58 of the remaining patients and identified for 52.

When several units were available for a patient, we selected the one that best matched the patient's HLA haplotypes and contained an optimal number of nucleated cells. In choosing among units with one HLA incompatibility, mismatches at class II MHC loci were considered as important as class I mismatches; when possible, mismatches within the same cross-reacting group of HLA antigens were preferred to major incompatibilities. When the donor unit and the potential recipient differed by two HLA antigens, more than one unit was usually available. In these instances, the results of high-resolution typing of HLA-DRB1 were used to find the unit with the closest match. In all other cases, high-resolution typing was analyzed retrospectively. After identification of a suitable placental blood unit, confirmatory HLA typing of the patient and the prospective unit was performed. A test sample from the unit that was transplanted into the one patient with Lesch–Nyhan disease was assayed for hypoxanthine phosphoribosyltransferase activity by Dr. William Nyhan. None of the transplanted placental-blood units were depleted of red cells, reduced in volume, or depleted of T cells before cryopreservation. All transplanted units were negative for human immunodeficiency virus (HIV), hepatitis A, B, and C, and human T-cell lymphotropic virus type I (HTLV-I) by standard blood-bank screening tests. None of the units, nor the mothers of the donors, were positive for IgM antibody to cytomegalovirus (CMV).

Transplantation Procedure

Cryopreserved units of placental blood were transported to the study center in a shipping container cooled by liquid nitrogen in vapor phase and stored in liquid nitrogen. For the first three transplantations, the unit of blood was thawed at the bedside and infused intravenously over a period of 10 to 15 minutes. For subsequent transplantations, the unit was thawed in the laboratory and washed with 10 percent dextran 40 (Baxter, Glendale, Calif.) and 5 percent human albumin before infusion.¹⁶ A nucleated-cell count, ABO and Rh typing, a test of cell viability, bacterial and fungal cultures, an assay for hematopoietic progenitor cells, and a CD34+ cell count were performed on a sample from each thawed unit at the time of infusion (Table 1).

View this table: Table 1. Characteristics of Placental-Blood Grafts.

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Preparative Regimens and GVHD

Table 2 lists the preparative regimens and details of prophylaxis against GVHD. Patients two years of age or older who had leukemia received hyperfractionated total-body irradiation (150 cGy twice a day for nine doses), melphalan, and antithymocyte globulin. Busulfan was given instead of total-body irradiation to patients under two years of age who had leukemia. Blood levels were measured after the second of 16 doses to achieve a steady-state concentration of 600 to 900 ng per milliliter, and doses 8 through 16 were modified if necessary. Patients with conditions other than leukemia were treated with other regimens, as



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TABLE 1. CHARACTERISTICS OF PLACENTAL-BLOOD GRAFTS.

PATIENT No.	ABO/RH TYPE	VOLUME COLLECTED	VOLUME INFUSED	No. OF NUCLEATED CELLS CRYOPRESERVED*	No. OF NUCLEATED CELLS INFUSED	No. OF COLONY- FORMING UNITS INFUSED	No. OF CD34+ CELLS INFUSED
	patient/donor	ml		$\times 10^{-7}/\text{kg}$		$\times 10^{-4}/\text{kg}$	$\times 10^{-6}/\text{kg}$
1	O+/O+	98	148	4.6	2.7	2.16	3.65
2	O+/O-	48	123	4.8	3.7	6.29	ND†
3	A-/A+	44	108	0.7	0.7	0.63	ND†
4	A+/O+	88	155	6.0	6.8	22.40	1.36
5	A-/O+	48	152	2.8	2.1	4.41	2.10
6	A+/O+	91	90	5.4	4.8	20.00	1.35
7	B+/O+	63	125	1.6	1.3	2.34	0.70
8	A+/A-	53	72	2.7	2.1	3.60	0.44
9	A+/O+	43	51	2.1	2.4	3.80	0.63
10	A+/A+	65	111	2.0	1.5	2.70	0.38
11	A+/A+	59	93	5.5	2.0	4.20	0.17
12	B+/A+	51	75	2.3	1.6	1.30	0.05
13	O+/B+	128	129	6.3	3.7	3.80	2.90
14	A+/O+	68	70	9.0	5.4	3.20	8.93
15	A-/B+	63	81	2.6	3.0	9.90	1.43
16	B+/O+	104	122	4.2	3.8	0.23	0.72
17	A+/AB-	52	55	4.1	2.4	3.10	1.92
18	A+/A+	214	56	7.8	4.3	15.05	1.72
19	B+/B+	128	51	13.1	7.4	8.14	1.48
20	A+/A+	101	51	16.8	9.8	6.86	2.50
21	B+/B+	107	98	4.2	3.7	2.96	0.95
22	O+/A+	109	50	14.0	11.0	13.30	2.64
23	A+/A+	70	36	7.9	4.4	1.10	1.76
24	B+/B+	40	60	1.47	0.7	0.55	2.76
25	O+/A+	108	98	1.80	1.1	1.48	1.14
Mean		82	90	5.38	3.70	5.74	1.81
Median		68	90	4.24	3.00	3.60	1.43
SD		39	35	4.24	2.67	6.00	1.82

*Conventional thawing techniques were used for Patients 1, 2, and 3, and dextran-albumin thawing techniques for Patients 4 through 25.



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TABLE 4. HLA TYPING OF DONORS AND RECIPIENTS.*

PATIENT No. AND BLOOD UNIT	CLASS I (SEROLOGIC TYPING)		CLASS II (LOW-RESOLUTION TYPING)	CLASS II (HIGH-RESOLUTION TYPING)	No. of MISMATCHED ANTIGENS	
	A	B	DR	DRB1	LOW RESOLUTION	HIGH RESOLUTION
	antigens		gene groups	genes		
Patient 1	2,29	44,60	4,7	0401,0701		
Blood unit	2,29	44,50	4,7	0407,0701	1	2
Patient 2	2,25	50,57	4,7	04xx1,0701		
Blood unit	2,26	50,57	blank,7	0701,0701	1	2
Patient 3	2,28	44,51	8,11	0801,1101		
Blood unit	2,28	44,51	7,11	0701,1101	1	1
Patient 4	2,blank	blank,44	12,13	1201,1301		
Blood unit	2,blank	41,44	12,13	1201,1302	1	2
Patient 5	11,26	35,70	2,4	1503,0401		
Blood unit	11,26	38,70	2,4	1501,0402	1	3
Patient 6	2,blank	57,62	2,7	1501,0701		
Blood unit	2,blank	57,44	2,7	1601,0701	1	2
Patient 7	24,28	39,60	4,blank	0403,0407		
Blood unit	24,28	39,61	4,blank	0411,0407	1	2
Patient 8	2,32	7,blank	1,2	0101,1501		
Blood unit	2,1	7,blank	1,2	0103,1501	1	2
Patient 9	2,3	7,18	7,11	0701,1101		
Blood unit	2,3	7,50	7,11	0701,1101	1	1
Patient 10	1,30	13,37	7,11	0701,1103		
Blood unit	1,30	13,37	7,11	0701,1103	0	0
Patient 11	11,31	35,40	4,blank	0404,blank		
Blood unit	11,31	35,58	4,blank	0402,blank	1	2
Patient 12	2,3	7,58	13,blank	1301,1303		
Blood unit	2,3	7,39	2,8	0801,1501	3	3
Patient 13	28,30	42,52	3,8	0302,0804		
Blood unit	28,30	7,42	3,8	0302,0804	1	1
Patient 14	2,blank	27,50	2,7	1501,0701		
Blood unit	2,blank	27,50	7,blank	0701,blank	1	1
Patient 15	11,24	14,51	1,11	0102,1104		
Blood unit	11,24	14,51	1,13	0102,1301	1	1
Patient 16	1,2	8,60	3,4	0301,0404		
Blood unit	1,2	8,60	3,4	0301,0404	1	1

described in [Table 2](#). Prophylaxis against GVHD consisted of cyclosporine alone for the two patients with Fanconi's anemia. A combination of methotrexate, cyclosporine, and methylprednisolone was given to the first 11 patients who did not have Fanconi's anemia. Since no case of severe GVHD occurred in these 11 patients, the regimen was reduced to cyclosporine alone in 2 patients and cyclosporine and high-dose methylprednisolone in 10 patients. Methotrexate was given in a dose of 15 mg per square meter of body-surface area on day 1, followed by 10 mg per square meter on days 3 and 6. Leucovorin (15 mg per square meter) was given 24 hours after each dose of methotrexate. The dose of methylprednisolone was 10 mg per kilogram of body weight on days 5 to 7, 5 mg per kilogram on days 8 to 10, 3 mg per kilogram on days 11 and 12, and 2 mg per kilogram on days 15 to 17; thereafter the dose was tapered 10 percent per week. The patients continued to receive a full dose of cyclosporine until a point between day 180 and day 270 after transplantation, after which the dose was tapered by 10 percent per week.

View this table: [Table 2. Preparative Regimens and Prophylaxis against GVHD.](#)

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Supportive Care

All patients were kept in reverse isolation under high-energy particulate air filtration. A parent roomed with each child. Prophylaxis with trimethoprim-sulfamethoxazole against *Pneumocystis carinii* was initiated before transplantation. Broad-spectrum antibiotic therapy was instituted at the time of the first episode of neutropenic fever and continued until the absolute neutrophil count exceeded 500 per cubic millimeter for two days. All patients received intravenous amphotericin B (0.25 mg per kilogram per day) from day 0 (the day of transplantation). The dose of amphotericin B was increased to 1 mg per kilogram per day if fever persisted for three days after the institution of antibiotic therapy. Patients received transfusions of leukocyte-depleted, irradiated, packed red cells and platelets to maintain platelet counts equal to or greater than 20,000 per cubic millimeter and hematocrit values greater than 27 percent during the first four weeks after transplantation. Filgrastim (10 µg per kilogram) was administered daily from day 0 until the absolute neutrophil count was 10,000 or more per cubic millimeter for three consecutive days or more than 2000 per cubic millimeter for two weeks. Patient 23 had polymicrobial sepsis at the time of preparation for transplantation and was supported with irradiated, filgrastim-mobilized parental granulocytes for the first 10 days after transplantation.

Intravenous immune globulin (Gamimune N, 10 percent, Cutter Biologicals, Elkhart, Ind.) was administered to each patient at a dose of 500 mg per kilogram weekly through day 100 and then once every two weeks or monthly during year 1. Patients who had IgG anti-CMV antibodies before transplantation or who received grafts that were CMV-positive (as determined by culture of the infants' saliva¹⁸) received ganciclovir through day 100. The presence of IgG anti-CMV antibodies alone in the infant-mother pair was not considered a risk factor for transmission of CMV through the placental-blood graft. Documented CMV infection that occurred after transplantation was treated with therapeutic doses of ganciclovir (5 mg per kilogram per dose, given twice a day) and intravenous immune globulin every other day for three weeks.¹⁹

Study End Points

To evaluate engraftment, the primary end point was the number of days required for the absolute neutrophil count to reach 500 per cubic millimeter. Secondary end points included the demonstration of megakaryocytes in the bone marrow and the number of days needed for the platelet count to remain above 20,000 per cubic millimeter and the hemoglobin level to stay above 10 g per deciliter without transfusion. Chimerism was evaluated by fluorescence in situ hybridization for the X chromosome in sex-mismatched transplants, or DRB1 allele-specific hybridization in cases in which the patient and donor differed at HLA-DR. These tests were performed 28 to 35, 100, 180, and 360 days after transplantation. Complete chimerism was inferred when all cells in the marrow and peripheral blood of the patient were of donor origin, whereas mixed chimerism was defined as the simultaneous presence of both donor and host cells. Primary graft failure was defined as a failure to reach a white-cell count of 500 per cubic millimeter or an absolute neutrophil count of 200 per cubic millimeter within 30 days of transplantation or a continued need for platelet transfusions for more than 100 days after transplantation in the absence of a frank leukemic relapse. Immunologic studies included assays for lymphocyte proliferation in response to plant mitogens (phytohemagglutinin, concanavalin A, and pokeweed mitogen) and tetanus toxoid and *Candida albicans* antigens; counting of the lymphocyte subgroups by fluorescence-activated cell sorting; and an assay of natural-killer-cell function by the measurement of lysis of the K562 cell line.

GVHD

GVHD was scored according to standard criteria.² During the first month after transplantation, all the patients were evaluated daily. After discharge from the hospital, patients were seen twice weekly during the second month after transplantation, weekly during the third through sixth months, and then quarterly. GVHD was documented histopathologically. DNA probes for maternal HLA genes were used to seek grafted cells derived from the donor's mother in all biopsy samples. GVHD (of grade II or higher) was treated with a high dose of methylprednisolone for three to seven days, after which the dose was tapered.

Statistical Analysis

The probability of event-free survival was calculated by Kaplan-Meier analysis.²⁰ Data on patients were censored at the first adverse event, such as death due to treatment-related toxicity, graft failure, relapse, or death from other causes. Correlations between the numbers of nucleated, hematopoietic progenitor, and CD34+ cells infused and the length of time to engraftment were expressed as the linear correlation coefficient (R^2) and evaluated by the t-test with SPSS for Windows software (SPSS, Chicago).

Results

Patient Characteristics

Twenty-five patients received transplants from August 1993 through November 1995 (Table 3). Searches for matched, unrelated bone marrow donors failed to identify such a donor for 17 of 22 patients. The median weight of the patients was 19.4 kg (range, 7.5 to 79.0) and the median age was 7.0 years (range, 0.8 to 23.5). Nineteen patients had malignant diseases and four had nonmalignant conditions; in two patients (Patients 8 and 23), a primary nonmalignant condition had converted to malignant disease. In nine patients,

prior infection with CMV was evidenced by IgG anti-CMV antibody titers of 1:8 or higher. Two patients with acute nonlymphocytic leukemia underwent transplantation during a relapse after they had undergone autologous bone marrow transplantation.

View this table: **Table 3.** Characteristics of the Patients.

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Selection of Units of Placental Blood for Transplantation

Units of placental blood were initially matched to the patient's HLA phenotype by serologic typing for class I HLA antigens and low-resolution DNA typing for class II HLA alleles. By these means, 1 of the 25 units we transplanted was matched for six of six HLA antigens, 20 were matched for five of six, 3 for four of six, and 1 for three of six. Subsequently, all the patients and units of placental blood were typed for HLA-DRB1 by high-resolution DNA hybridization with group-specific polymerase-chain-reaction primers and allele-specific oligonucleotide probes. This second genetic analysis revealed the following distribution of HLA matches: 1 donor-recipient pair at six loci, 9 pairs at five loci, 11 pairs at four loci, and 4 pairs at three loci (Table 4). When a matched unit was available, the median time to its identification was 3 days, and the median time to transplantation 102 days. When a continued search was required to find a suitable unit, the average time to identification was 18 days (range, 0 to 128), and the time to transplantation 115 days (range, 12 to 291).

View this table: **Table 4.** HLA Typing of Donors and Recipients.

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Engraftment

There was evidence of myeloid engraftment in 23 of the 25 patients. In one patient (Patient 3), the infused cells failed to engraft, but spontaneous reconstitution with autologous cells occurred 65 days after transplantation. Two patients (Patients 9 and 12) had persistent leukemia (one of them had evidence of myeloid engraftment), and the absolute neutrophil count never rose above 500 per cubic millimeter. In the remaining 22 patients, the absolute neutrophil count reached 500 per cubic millimeter in a median of 22 days (range, 14 to 37). Platelet transfusions became unnecessary in a median of 56 days (range, 35 to 89) in 16 patients who could be evaluated. Platelet counts of 50,000 and 100,000 per cubic millimeter were reached by a median of 82 and 115 days, respectively. Red-cell transfusions could be stopped after a median of 55 days (range, 32 to 90). Seven patients died of relapse (Patient 9), regimen-related toxicity (Patients 7 and 17), or infection (Patients 8, 11, 19, and 21) while still dependent on platelet or red-cell transfusions. All surviving patients were complete chimeras as of the most recent follow-up in May 1996.

Cell Dose and Speed of Engraftment

The number of nucleated cells infused per kilogram of the patient's body weight correlated with the rate of

myeloid engraftment ($P=0.002$; data not shown). There was a trend for the time to myeloid or platelet engraftment to increase with the dose of clonogenic precursors or CD34+ cells, but the correlations were not statistically significant. After observing delayed recovery of neutrophils in the first three patients, we began to use a new thawing technique that increases cell viability in vitro.¹⁶ In patients infused with these "washed" units of placental blood, myeloid engraftment was accelerated ([Figure 1](#)), but platelet and red-cell engraftment was not affected.

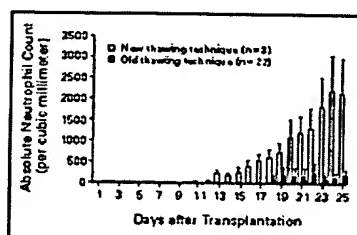


Figure 1. Relation of Neutrophil Recovery to Thawing Method.

Values shown are means \pm SD.

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GVHD

Twenty-one of the 25 patients could be evaluated for GVHD. Four patients did not have GVHD; eight patients had grade I GVHD, involving only the skin; and seven patients had grade II GVHD, involving the skin and gut. In two patients (Patients 13 and 16, who were recipients of grafts that were mismatched at HLA-B and at HLA-B and HLA-DRB1, respectively), grade III GVHD, involving the skin and gut, developed. No patient had acute grade IV GVHD. In two patients (Patients 5 and 16), chronic GVHD limited to the liver or skin developed 19 and 7 months after transplantation. There was no correlation between the incidence or extent of GVHD and the degree of HLA mismatching (data not shown). Maternal cells from the donor unit were not found in the tissue-biopsy specimens from any patient with GVHD. All patients with GVHD responded to treatment with high doses of methylprednisolone, and none required second-line therapy for steroid-refractory GVHD.

Immunologic Reconstitution

In vitro responses to T-cell and B-cell mitogens were detectable in 13 patients with engraftment who were studied within three months of transplantation ([Table 5](#)). The absolute lymphocyte counts were greater than 500 per cubic millimeter, but the CD4:CD8 ratios were inverted in all patients studied for the first six months after transplantation. Natural-killer-cell function was normal in six patients tested two to three months after transplantation.

View this table: [Table 5. Immunologic Reconstitution.](#)

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Survival



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TABLE 5. IMMUNOLOGIC RECONSTITUTION.

PATIENT No.	POST- TRANSPLANTATION DAY	MITOGEN*			ABSOLUTE LYMPHOCYTE COUNT	CD4:CD8 RATIO
		PHA	CON A	PWM		
		counts per minute			cells/mm ³	
1	60	141,124	62,712	53,226	1,408	0.33
2	95	63,561	53,968	26,011	2,176	2.25
	192	59,991	42,024	26,817	2,279	0.94
	380	245,573	279,177	109,366	2,262	1.46
	820	153,606	280,548	85,583	5,568	1.93
4	118	128,537	139,027	81,733	495	0.80
	245	105,815	122,060	40,860	690	3.99
	361	163,680	417,051	276,527	986	2.07
	418	298,166	239,396	33,580	1,350	2.04
5	84	92,050	90,444	2,085	1,463	0.33
	176	101,962	53,207	22,033	2,272	0.60
	260	66,128	35,254	2,291	9,570	1.05
	358	111,070	59,867	3,567	11,900	1.42
	512	276,131	324,249	59,229	4,620	0.78
6	95	90,539	81,933	17,245	469	0.37
	158	82,141	114,941	46,765	1,504	0.67
10	71	58,656	76,793	8,705	792	0.54
	204	166,290	116,710	47,095	NA	0.64
	428	284,755	237,501	143,944	2,624	1.92
13	95	87,094	79,724	14,797	266	0.58
14	60	22,617	39,788	1,614	4,020	0.11
	103	47,114	52,213	30,078	3,000	2.47
	299	307,475	208,247	21,369	4,092	0.78
15	54	248,565	254,876	13,328	510	2.33
	98	146,864	90,537	10,900	900	1.42
16	190	192,657	197,215	52,592	736	0.87
20	187	264,935	189,613	156,324	2,720	1.93
22	95	61,829	58,982	4,280	940	2.31
23	53	146,650	74,027	137,584	648	1.51

*PHA denotes phytohemagglutinin, Con A concanavalin A, and PWM pokeweed mitogen. The respective mean (\pm SD) normal values in children from the Duke University Medical Center Clinical Pediatric Immunology Laboratory are $240,684 \pm 125,036$, $214,779 \pm 112,333$, and $157,469 \pm 60,364$.

As of June 1996, 12 of the 25 patients had survived event-free for 7 to 32 months after transplantation (Table 6), for an event-free-survival rate of 48 percent. Seven of the 19 patients undergoing transplantation for malignant conditions and 5 of the 6 with nonmalignant conditions had survived event-free with Karnofsky scores above 90, with a median follow-up of 12 1/2 months (range, 7 to 32). Only one of the eight patients who underwent transplantation while in remission and survived for more than 100 days had had a leukemic relapse. Thirteen of the 25 patients had died of infection, relapse, or toxic effects of treatment.

View this table: [Table 6. Engraftment, GVHD, and Survival in Patients Who Received HLA-Mismatched Placental Blood.](#)
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Discussion

In this study we found that hematopoietic stem cells in placental blood from unrelated donors engrafted and reconstituted hematopoiesis in more than half of a group of high-risk patients, nearly all of them children, with malignant and nonmalignant conditions. There was a complete match between the donor and the recipient for all class I HLA antigens and class II HLA alleles in only one case. Despite the HLA incompatibility, GVHD was mild (less than grade III) in all but two recipients. The substitution of melphalan for cyclophosphamide in patients with malignant conditions allowed adequate engraftment.^{22,23,24,25,26,27,28,29} Complete donor chimerism was achieved without total-body irradiation in nine patients who were prepared with chemotherapy alone. This experience is relevant for transplantation in younger children, who are at especially high risk for treatment-related neurotoxicity.³⁰ The recovery of platelets and red cells was later in all recipients of placental-blood grafts than in recipients of marrow grafts, perhaps because placental blood contains a higher proportion of immature hematopoietic progenitor cells than does bone marrow.^{31,32,33,34,35,36,37,38}

Engraftment occurred in patients who received as few as 6 million nucleated, HLA-disparate, placental-blood cells per kilogram, but we do not know whether this result in children can be applied to adults.^{31,32,33,34,35,36,37,38} We found a relation between the speed of myeloid recovery and the dose of infused cells, but the International Cord Blood Transplant Registry found no such correlation in recipients of placental blood from related donors.¹¹ This difference may be due to the uniformity of results a single center can obtain, or to the use of filgrastim in our patients. Although the number of CD34+ cells (presumably hematopoietic progenitor cells) in the graft correlates with myeloid and platelet engraftment in recipients of filgrastim-mobilized peripheral-blood progenitor cells, we did not find any such correlation.

All but one of the pairs of donors and recipients in this series differed by one to three HLA antigens. Nevertheless, primary graft failure occurred only in patients who underwent transplantation during leukemic relapse, and severe acute or chronic GVHD was not observed. By contrast, in 462 patients undergoing bone marrow transplantation from HLA-matched, unrelated donors, the probability of grade III or IV acute GVHD was 47 percent overall and 30 percent for patients less than 18 years of age; for chronic GVHD, the probability was 55 percent.² Contamination of placental blood by maternal cells is a potential

cause of serious GVHD,^{39,40,41,42,43} but our results do not support this possibility. The observation that placental T cells mount less of a graft-versus-host response than bone marrow from unrelated donors in HLA-mismatched recipients has yet to be explained, but it parallels observations made in vitro and in animal studies.⁴⁴ Another unsolved problem is whether the lower incidence of GVHD indicates a decrease in graft-versus-leukemia activity and thus an increase in the risk of leukemic relapse. Our data are too premature to answer this question.

Banked placental blood has potential advantages over bone marrow from adults. Placental blood is less likely than adult bone marrow to contain viruses; the storage of placental blood reduces procurement time to one or two weeks, substantially less than the four to six months typically needed to find an unrelated bone marrow donor; and with placental blood the time required to prepare the patient for transplantation is usually short.

A major disadvantage of placental-blood transplantation is that only one unit is available for each transplantation procedure. Ex vivo expansion of stem cells and progenitor cells might circumvent this problem. A second possible disadvantage is the unwitting transmission of a genetic disease affecting hematopoietic cells. Placental blood can be tested for common hematologic diseases, such as hemoglobinopathies, before banking or transplantation, and the family history can reveal the possibility of an inherited disorder. Nevertheless, extremely rare genetic diseases are unlikely to be revealed by the family history, and laboratory testing for them may be impractical or impossible.

In summary, we have demonstrated that partially mismatched placental blood from unrelated donors can provide an alternative source of stem cells for hematopoietic reconstitution. The use of placental blood that differed from the recipient's by one to three HLA alleles resulted in 100 percent donor chimerism, generally treatable GVHD, and immune reconstitution.

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Source Information

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